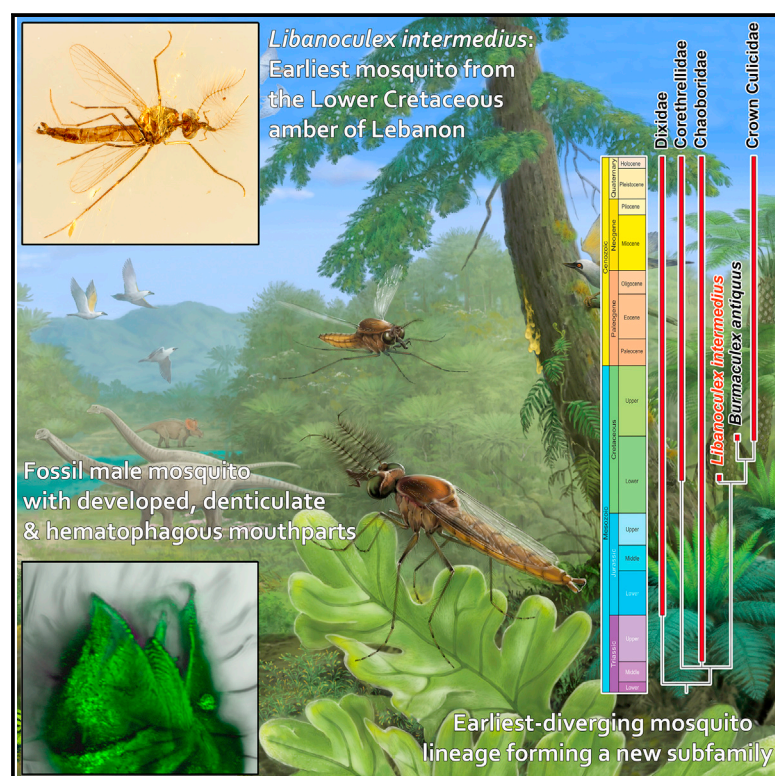


Current Biology

The earliest fossil mosquito

Graphical abstract



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In brief

Azar et al. describe the earliest known fossil mosquito from Lower Cretaceous Lebanese amber. A new, early diverging subfamily of mosquitoes (Culicidae), the species is represented by two conspecific males with well-developed, denticulate, and hematophagous-type mouthparts, indicating that these males were likely blood feeders.

Highlights

- Earliest-diverging lineage and oldest occurrence of mosquitoes
- Male mosquito with well-developed, denticulate, and hematophagous-type mouthparts
- New extinct subfamily of mosquitoes



Report

The earliest fossil mosquito

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SUMMARY

Female mosquitoes are among the most notorious blood-feeding insects, sometimes causing severe allergic responses or vectoring a variety of microbial pathogens.^{1,2} Hematophagy in insects is likely a feeding shift from plant fluids, with the piercing-sucking mouthparts serving as suitable exaptation for piercing vertebrates' skin. The origins of these habits are mired in an often-poor fossil record for many hematophagous lineages,^{3,4} particularly those of sufficient age, as to give insights into the paleoecological context in which blood feeding first appeared or even to arrive at gross estimates as to when such shifts have occurred. This is certainly the case for mosquitoes, a clade estimated molecularly to date back to the Jurassic.⁵ The known Mesozoic Culicidae are Late Cretaceous, assigned to the modern Anophelinae or to the extinct Burmaculicinae, sister to other Culicidae, all with mouthparts of a modern type. Here, we report the discovery, in Lower Cretaceous amber from Lebanon, of two conspecific male mosquitoes unexpectedly with piercing mouthparts, armed with denticulate sharp mandibles and laciniae. These male fossils were likely hematophagous. They represent a lineage that diverged earlier than Burmaculicinae, extending the definitive occurrence of the family into the Early Cretaceous and serving to narrow the ghost-lineage gap for mosquitoes.

RESULTS

Insect hematophagy on vertebrates, including humans, is an important problem in public health. This type of micro-predation can cause serious illness.^{1,2} Hematophagy in many insects is likely the result of shifts in feeding from plant fluids such as nectar, with the piercing-sucking mouthparts of many fluid-feeding groups serving as a suitable exaptation for the piercing of vertebrate skin and vice versa. For instance, the exclusively hematophagous fleas seem to have diverged from nectar-feeding Mecoptera.⁶ The situation is rather particular among the culicomorph flies that are nectar feeding, but some groups have developed blood feeding in relation to increased egg production by females, and they seem to form a complex mosaic of changes among culicomorph flies. For example, it seems that the oldest representatives of the nectar-feeding Chironomidae were once hematophagous.⁷ The fossil record reflecting these changes is often poor for many hematophagous insect lineages,^{3,4} particularly those of sufficient age, as to give insights into the paleoecological context in which blood feeding first appeared—or even to arrive at gross estimates as to when such a shift may have occurred. All extant (and likely fossil) female mosquitoes (Culicidae) are hematophagous and nectarivorous, whereas extant species of their sister group, Chaoboridae, are

nectar feeding. Thus, a shift from strict nectarivory to partial hematophagy has occurred, but it is currently not possible to determine whether the earliest of stem-group Culicidae were hematophagous or not. Fossil mosquitoes are largely confined to Cenozoic deposits and comparatively modern taxa. Occurrences for Mesozoic Culicidae are rare, with only three definitive records, two from Upper Cretaceous amber deposits (Canada, Myanmar), along with an indeterminate putative culicid from the upper Campanian of Arizona. These fossils have been assigned to either the modern subfamily Anophelinae or to the extinct subfamily Burmaculicinae, sister to all other Culicidae. Molecular estimates for the initial split and divergence of the crown-group mosquitoes have recovered an Early Jurassic age (ca. 197.5 million years [Ma]).⁵ Accordingly, these estimates imply mosquitoes' ghost lineage spanning nearly 97 Ma.⁵ The related family Chaoboridae has fossil occurrences dating from the Middle to the Late Triassic.⁸ Nonetheless, if there existed an extensive stem-lineage before the appearance of crown-group Culicidae, the culicine-anopheline divergence could have taken place much later in the Jurassic or even in the Early Cretaceous, particularly if the phylogenetic placement of the extinct Burmaculicinae as sister to the crown group (Culicinae + Anophelinae) is accurate. Critical to the ongoing debate is the discovery of new fossil material predating the “mid” Cretaceous.

Here, we report the discovery of two conspecific male mosquitoes with piercing mouthparts armed with denticulate sharp mandibles and laciniae, preserved in Lower Cretaceous amber from Lebanon, extending the definitive occurrence of the family into the Early Cretaceous. Phylogenetic analysis indicates that the new fossil represents a lineage of mosquitoes that diverged earlier than Burmaculicinae, narrowing the ghost-lineage gap for mosquitoes and providing glimpses into Mesozoic culicid paleodiversity. This discovery also suggests that not only were the earliest female mosquitoes hematophagous but males were also in some cases.

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Systematic paleontology

Order Diptera Linnaeus, 1758

Family Culicidae Billberg, 1820

Libanoculicinae subfam. nov.

urn:lsid:zoobank.org:act:148B3C46-BB59-4C37-AC99-D5F41D41509C

Type genus

Libanoculex gen. nov.

Diagnosis

Culicomorpha male (female unknown) (Figures 1A and 1B): head with developed proboscis (Figure 1C), slightly longer than 1/3 length of antenna; mandibles exceptionally sharp, denticulate, and elongate, extending to apex of hypopharynx; lacinia denticulate (Figures 1D–1F); maxillary palpus without scales, maxillary palpomere III longest, slightly longer than palpomere V; setae on length of flagellomeres 0.25× length of those of basal ring; clypeus with sparse setae; scales present along apical and posterior margins of forewing (Figures 1G and 1H), wing veins lacking scales; meron continuously sclerotized with dorsal margin abutting katepimeron (Figure 1J).

Libanoculex gen. nov.

urn:lsid:zoobank.org:act:D1608747-362D-4E5F-B094-01CEDE7B2585

Type species

Libanoculex intermedius sp. nov.

Etymology

The generic name is a combination of the Latin *Libanus*, meaning “Lebanon,” and the generic name *Culex* L. (Latin, meaning “gnat” or “mosquito”). Gender of the name is masculine.

Diagnosis

As for the subfamily (as above).

Libanoculex intermedius sp. nov.

Figures 1, 2, and S1.

urn:lsid:zoobank.org:act:C20EBC52-D667-4E81-B707-FAECB5EE3CBE

Etymology

The specific epithet is the Latin adjective *intermedius*, meaning “intermediate” or “between,” and refers to the early diverging position of the fossil relative to other Culicidae and its unique combination of culicid apomorphies and symplesiomorphies with Chaoboridae.

Type material

Holotype 1717A (a nearly complete male, in amber) (Figures 1A and 1B), Paratype 825A (a nearly complete male, in syninclusion with a Thysanoptera in amber), accessioned at the Natural

History Museum of the Lebanese University, Faculty of Sciences II, Fanar, Lebanon.

Outcrop and horizon

Mdeirij-Hammana outcrop (33°48′13″N, 35°43′45″E, elev. 1,340 m), Caza (= District) Baabda, Central Lebanon, lower Barremian.^{9,10}

Diagnosis

As for the genus (as above).

Description

Male (based on holotype). Body and appendages clear reddish brown; body length from top of proboscis to tip of abdomen 2.45 mm (Figures 1A, 1B, S1A, and S1B; Video S1). Head width 0.50 mm (Figures 2A, S1C, and S1D; Videos S2 and S3); vertex with two converging lines of long setae, disposed on either side of frontal suture in a V-shape; post-occipital suture with dense long setae; compound eyes reniform with facets of equal size, eyes separated by 0.14 mm; antenna bushy (Figure S1C), longer than maxillary palpus and proboscis (Figure S1D), 1.27-mm long, with 13 flagellomeres, flagellum length 1.19 mm; flagellomeres XII and XIII longest (Figure 2B), flagellomere XII (0.22 mm) longer than flagellomere XIII (0.17 mm), each flagellomere with flagellar whorl made of a ring of long curved setae situated at basal third, except for flagellomeres XII and XIII having a whorl more basal and shorter curved setation along whole length of flagellomere; scape short, partially hidden by large, cup-shaped pedicel; clypeus setose, elongate with triangular-shaped tip, 2.27-mm long; labrum straight, exposed, with sharply pointed apex; proboscis bearing numerous setae, especially on ventral surface near tip on labellum; labellum comprising two incompletely separated sclerites; lacinia, mandible, and hypopharynx sharply pointed; mandible and lacinia bearing small denticulation (Video S4); maxillary palpus pentamerous, 0.36-mm long, slightly shorter than proboscis (0.40 mm), lengths of palpomeres I–V, respectively, 0.025, 0.043, 0.112, 0.075, and 0.106 mm; palpomere II bearing a long sensillum chaeticum on distal third of inner surface, palpomere III longest, bearing a sensillum (capitate peg) on basal fourth of inner surface (Figure S1E); palpomere IV with one or two sensilla chaetica on its basal inner surface.

Scutellum arched; forewing elongate (Figure 2C), 1.63-mm long, 0.46-mm wide; humeral vein reaching wing margin at 0.29 mm apically; subcostal vein (Sc) meeting costal vein (C) on wing margin slightly beyond r-m crossvein, at 1.1 mm from wing base; R1 reaching C 1.37 mm apically, beyond fork of R2 + 3; R2 and R3 reaching wing margin at 1.53 and 1.63 mm from wing base, respectively; R4 + 5 reaching wing apex; M1 + 2 bifurcating at 1.26 mm from wing base; M1 and M2 reaching wing margin posteriorly at 1.56 and 1.41 mm from wing base, respectively; M4 and CuA meeting wing margin at 1.30 and 1.14 mm; anal vein (A) reaching wing margin before fork of CuA; posterior and apical portions of C bearing a row of fringe scales and short secondary scales (Figure S1F); halteres 0.23-mm long (Figure 2D); tarsi pentamerous; tarsomeres I–IV with apical spines; each leg bearing a pair of curved ungues with two or more long, preapical teeth; pulvilli or empodium or prong present.

Abdomen 0.90-mm long; male genitalia with gonocoxite 0.17-mm long and 0.08-mm wide at widest point; gonostylus 0.12-mm long, a rather large structure looking like parameres present (Figures 1K and 2E).

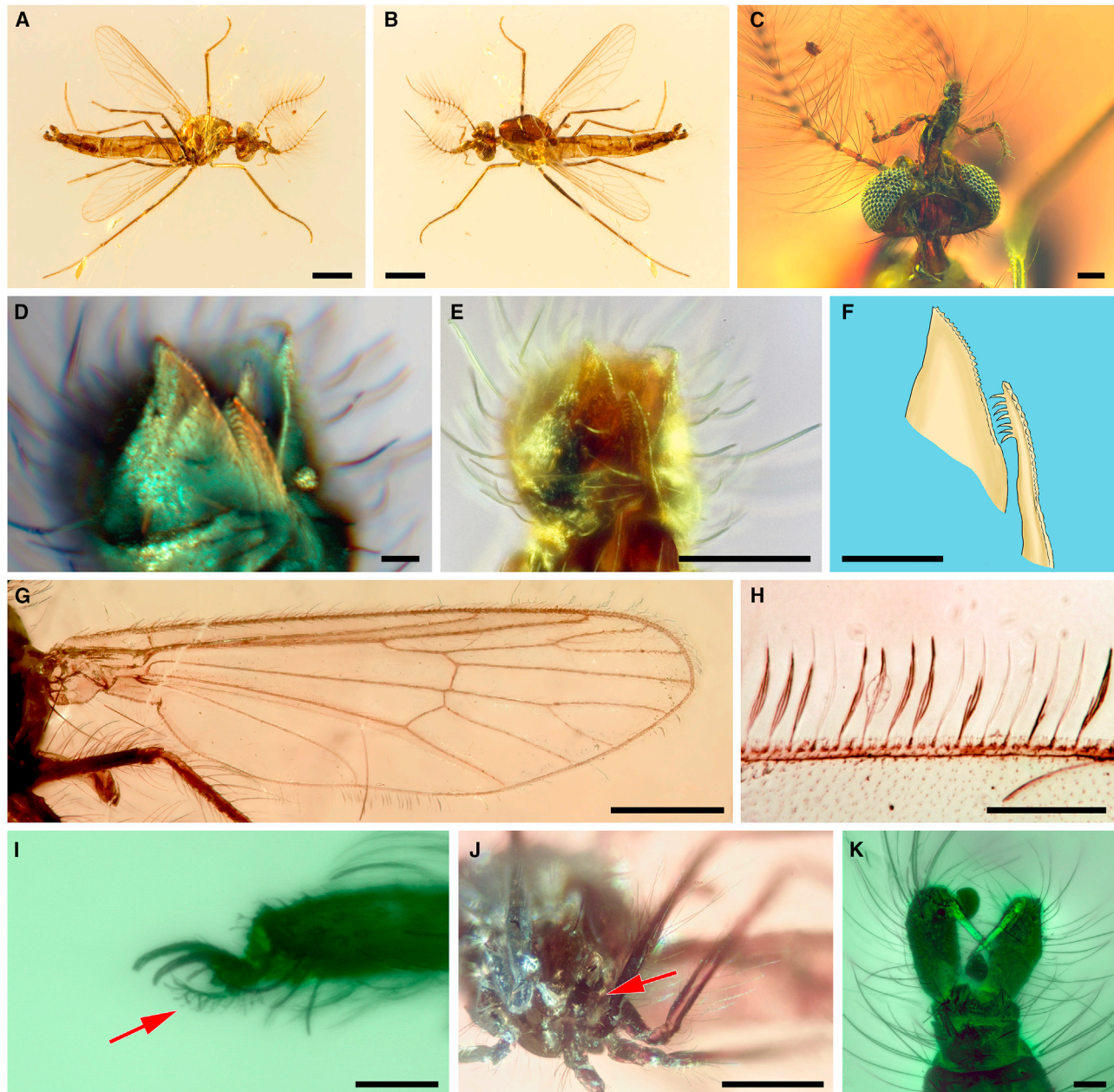


Figure 1. Holotype of *Libanoculex intermedius* gen. et sp. nov

(A) Ventral view of habitus; scale bar, 500 µm.

(B) Dorsal view of habitus; scale bar, 500 µm.

(C) Head, ventral view; scale bar, 100 µm.

(D) Detail of mouthparts using a confocal microscope; scale bar, 10 µm.

(E) Detail of mouthparts using a light compound microscope; scale bar, 50 µm.

(F) Drawing of the apex of the mandible and lacinia; scale bar, 50 µm.

(G) Wing; scale bar, 300 µm.

(H) Detail of scales on posterior margin of wing; scale bar, 50 µm.

(I) Detail of tarsal claw, arrow indicates prong; scale bar, 20 µm.

(J) Detail of the thoracic wall, arrow indicates meron; scale bar, 300 µm.

(K) Male genitalia, ventral view; scale bar, 50 µm.

See also [Figure S1](#) and [Videos S1, S2, S3, and S4](#).

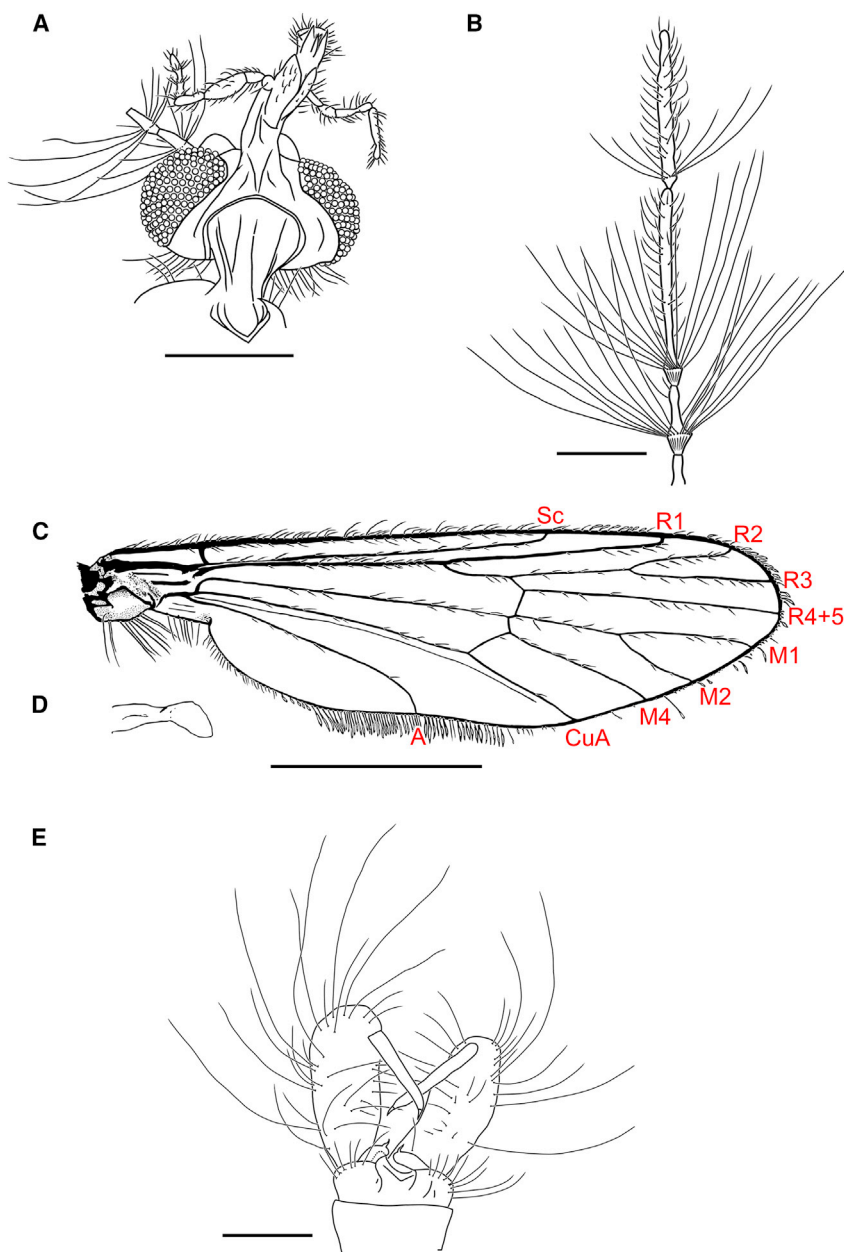


Figure 2. Drawings of *Libanoculex intermedius* gen. et sp. nov., holotype

(A) Head, ventral side; scale bar, 300 μ m.

(B) Apex of antenna; scale bar, 100 μ m.

(C) Wing; scale bar, 500 μ m.

(D) Halter; scale bar, 500 μ m.

(E) Male terminalia; scale bar, 10 μ m.

of flagellomeres” as an apomorphy, whereas the (Chaoboridae + Culicidae) have the elongate setae restricted to the basal ring¹² (Figure 1C). In the new fossil, there are few setae along the length of the flagellomeres, but these are 0.25 $\times$ the length of those of the basal ring. Therefore, as a putative symplesiomorphy, it provides no evidence of relationship. The characters of “clypeus has sparse setae,” the “[meso]femur is of equal or lesser diameter than those of fore- and hind legs,” “[meso]tibia with spur,” and the “posterior [surface] of [meta]tibia of adult with undifferentiated setae” (brackets indicate our corrections), also symplesiomorphic character states present in the (Chaoboridae + Culicidae). The only known synapomorphy of adult (Chaoboridae + Culicidae), concerns the male accessory gland,¹¹ a structure unobservable in the new fossil.

Culicidae have a long proboscis (apomorphy), whereas in Chaoboridae, the proboscis is considerably shorter. The new fossil has a rather elongate proboscis (Figure 1C), although it is still clearly shorter than those among extant Culicidae but longer than Chaoboridae. Its maxillary palpi are as short as those in Chaoboridae but shorter than in the Burmese amber fossil *Burmaculex antiquus*, the latter a stem-group culicid, whereas crown-group Culicidae have distinctly longer palpi. Extant Culicidae have a

DISCUSSION

The new fossil has the adult synapomorphy for Culicoidea, i.e., “adult male with anterior claw of each leg with a basal prong”¹¹ (Figure 1I). Within the superfamily, it lacks the two synapomorphies proposed for Dixidae (i.e., “R₁ extending very near or to wing apex and curved apically” and “R₂ + ₃ strongly arched”), excluding affinities with this family. Additionally, it possesses two of the synapomorphies established for the clade comprising (Corethrellidae + [Chaoboridae + Culicidae]), specifically, “adult with anterior anepisternum divided by sinuous suture into dorsal and ventral portions” and “posterior margin of adult wing with scales” (Figure 1H).

Extant adult Corethrellidae have “basal flagellomeres (generally 1–10) with additional elongate setae on length

bare clypeus, unlike Chaoboridae, the new fossil, and *B. antiquus*. Maxillary palpomere III of the new fossil is longer than others, but only slightly longer than palpomere IV, whereas it is disproportionately elongate in Culicidae and *B. antiquus*. The palpus of the new fossil lacks scales, unlike crown-group Culicidae. No adult characters were proposed as synapomorphies for all Chaoboridae,¹¹ rendering it difficult to attribute fossils to this family with confidence, especially those from the Mesozoic.

Two synapomorphies have been proposed for extant adult Chaoboridae, viz. “meron is largely membranous with only two small semicircular sclerotized areas along the anterior margin,”¹³ whereas in the new fossil, the meron is “continuously sclerotized with the dorsal margin abutting the katepimeron” (Figure 1J), as in other Culicomorpha. The second character

concerns the bases of the mandibles,¹³ which are not discernible in the new fossil. These putative synapomorphies “warrant further outgroup comparisons.”¹¹

A “proboscis of 1/3 the length of the antennae” is proposed as a character state grouping *B. antiquus* with extant Culicidae.¹ The proboscis of the Lebanese fossil is slightly longer than 1/3 the length of the antenna, which is distinctly longer than the proboscis of extant Chaoboridae, and matches well with the proposed synapomorphy for (Burmaculex + crown-Culicidae), suggesting that this character supports the placement of the Lebanese fossil within the stem-group Culicidae.

Other non-culicid Culicomorpha have a comparatively shorter palpomere III, more or less equal in length to or shorter than the combined lengths of palpomeres IV and V.¹ In the current fossil, maxillary palpomere III is slightly longer than palpomere IV, but shorter than the combined lengths of palpomeres IV and V, corresponding to the plesiomorphic condition in extant Chaoboridae.¹⁴

The mandible of the Lebanese fossil is a triangular but exceptionally sharp and elongate structure reaching the apex of the hypopharynx and bearing small denticles (Figures 1D–1F). In female *Corethrella*, the mandible is also denticulate, but it is distinctly broader and appears shorter than that of the Lebanese fossil because *Corethrella*’s mouthparts are shorter. It is even more weakly developed in males.^{13,15,16} In the chaoborid *Eucoethra*, the mandible is also elongate, extending beyond the apex of the labrum but rather looking like a scalpel, not denticulate, and not as sharp as the mandible of the Lebanese fossil.¹³ They are not functional in *Corethrella*. Nevertheless, it is less thin and acute in the new fossil than in extant Culicidae.¹³ In *Chaoborus* and *Mochlonyx*, the mandible is also scalpel-like and shorter than in *Eucoethra*, clearly not reaching the apex of the hypopharynx.^{11,14} The lacinia of the new fossil is formed as a sharp stylet, bearing small denticles apically (Figures 1D–1F), unlike the non-functional ones of extant Chaoboridae in which the lacinia is blade-like and at most spinose (in *Mochlonyx*).¹⁴ Corethrellidae also lack denticles on the lacinia.¹⁵ Such apical denticles on the lacinia are also present in the hematophagous representatives of Psychodidae, the clade (Simuliidae + [Chironomidae + Ceratopogonidae]), and Culicidae.^{17–20} The new fossil only shares symplesiomorphies with Chaoboridae and three putative synapomorphies with the stem-group Culicidae, i.e., proboscis of 1/3 the length of the antennae (Figure 1C), “mandible very sharp, denticulate, at least reaching apex of hypopharynx,” and “lacinia sharp and denticulate” (Figures 1D–1F). Accordingly, available evidence indicates that the fossil is a member of the culicid stem group, although diverging earlier than Burmaculicinae and thus the earliest evidence of mosquitoes. The Late Cretaceous genus *Pa-leoculicis* is difficult to place but appears to belong to crown-Culicidae, whereas the mid-Cretaceous *Priscoculex* is placed in Anophelinae, thus also in crown-Culicidae.²

Our phylogenetic analysis recovered a single parsimonious topology (Figure 3), with length 16 steps, consistency, and retention indices = 1 (homoplasy index = 0). The cladogram can be summarized as Dixidae (Corethrellidae + Chaoboridae + [*L. intermedius* (*B. antiquus* + crown-Culicidae)]), confirming the position of *L. intermedius* as sister to all other Culicidae. We found 96% bootstrap support for *L. intermedius* (*B. antiquus* + crown-Culicidae) and 92% for (*B. antiquus* + crown-Culicidae).

Libanoculex intermedius gen. et sp. nov. currently represents the earliest-diverging lineage and oldest occurrence of mosquitoes, extending the age of the greater culicid clade back by about 30 Ma. The dating at 197.5 Ma for divergence time estimates between the two extant culicid subfamilies⁵ could appear surprising if we consider that no representative of the crown group Culicidae is known in the Jurassic. But some Jurassic taxa, described on the basis of compression fossils, currently attributed to the Chaoboridae,²¹ could be in fact stem-group Culicidae. There are no synapomorphies for the adult stage of Chaoboridae, and therefore, one cannot be certain whether some of these Jurassic fossil “Chaoboridae,” represented by adults, might not belong to early lineage(s) of Culicidae.¹¹ The exceptionally tiny structures of the thorax and mouthparts that would potentially allow an attribution of some of these Jurassic fossils to the stem or the crown Culicidae are hardly discernible on amber inclusions with their life-like fidelity, and the situation is considerably worse for compression fossils.

The presence of exceptionally sharp and denticulate mandibles and laciniae in the male of *L. intermedius* suggests that these were hematophagous. It also has sensilla on maxillary palpomeres II–IV, sensilla chaetica on palpomeres II and IV, and capitate peg sensilla on palpomere III, all of which strongly resemble the capitate pegs and sensilla chaetica of extant *Anopheles* spp.^{22,23} The sensilla chaetica of Culicidae have been indicated as potentially sensitive to air currents, whereas the capitate pegs could be olfactory receptors sensitive to carbon dioxide. Accordingly, these likely served to aid the detection of females or of potential vertebrate hosts,²⁴ possibly further supporting a hematophagous behavior for the male of *Libanoculex intermedius*.

Such behavior would be surprising for a male mosquito, given that the extant males feed on nectar, plant sap, or honeydew.²⁵ Nevertheless, male hematophagy is known in extant Psychodidae^{3,26} and some cyclorrhaphous brachyceran flies. Critically, rare cases of hematophagy by male Culicidae have been reported,^{27,28} with an apparently toxic effect on the insect. Regardless, the occurrence of blood-feeding males in other lineages, as well as rare instances within Culicidae, suggest that it should perhaps not be all that alarming to find such feeding in a Cretaceous male mosquito.

Extant male culicids have reduced and shortened mandibles that are probably not active in piercing, unlike those of *Libanoculex intermedius*.^{29,30} A remarkable parallel situation occurs between the Cretaceous hematophagous (and probably nectarivorous) male Chironomidae versus the nectar-feeding modern species.³¹ The “origins” of the existence of hematophagous male Culicidae and Chironomidae during the Early Cretaceous remain unknown.

Hematophagous male psychodids have shorter mandibles than the females²⁰; thus, one might hypothesize that the female of *Libanoculex intermedius* could have had more developed mouthparts than the corresponding male, even if the mandibles in the extant non-bloodsucking chaoborids are similar in both sexes,¹⁴ suggesting the contrary. Only the discovery of the female of *Libanoculex intermedius* will resolve this and further paleobiological questions relating the role of mosquitoes for spreading vertebrate pathogens in the mid-Mesozoic.

Generally, culicomorphan flies that feed on nectar do not have denticulate mandibles and laciniae, even if some male Ceratopogonidae, whose biology is poorly known, show reduced

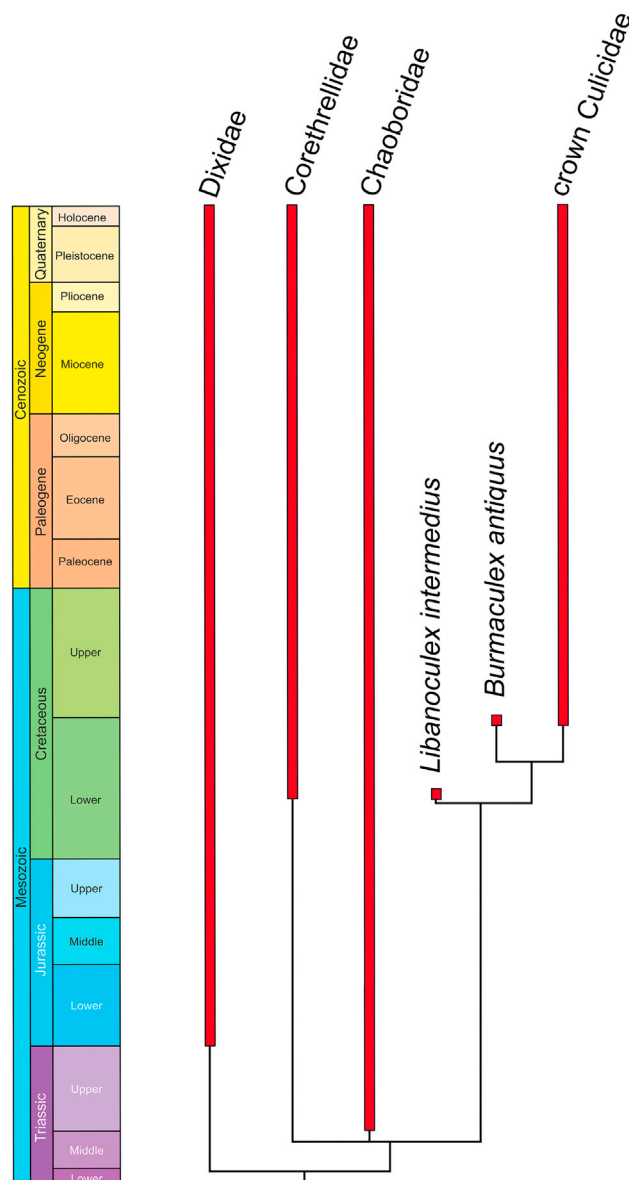


Figure 3. Phylogenetic single parsimonious cladogram confirming the position of *L. intermedius* as sister to all other Culicidae; CI = RI = 1, HI = 0

denticulation on their mandibles.^{32,33} Borkent¹⁶ has noted that “nematocerous Diptera with females that have finely toothed mandibles but smooth laciniae are all frog-biters.” This is the case in the families Corethrellidae, Psychodidae, and Ceratopogonidae. The presence of denticulate laciniae in *L. intermedius* would therefore imply that this fly attacked hosts with thicker skins.

Although only extant females feed on blood, females and males of these species also feed on nectar. The benefit for Cretaceous male mosquitoes to feed on blood could have been to increase their capacity to fly and successfully mate, as is the case in extant females, but a reason for why this behavior was subsequently lost in males remains unknown.

Amber of Lebanon was formed under an equatorial tropical forest situated in northeastern Gondwana on the shore of the Tethys during the Early Cretaceous, a period of considerable biotic turnover associated with the radiation of angiosperms. Lebanese amber inclusions often represent “missing links” between the ancient Jurassic fauna and the latter angiosperm-associated biota, and *L. intermedius* would appear to be another such example. Currently, the discovery of many new outcrops of Lebanese amber helps to meet the challenge of considerably enriching our knowledge of the past,¹⁰ and these outcrops are our best chance of relieving the itch to know more about biodiversity in the northeast of Gondwana during the Early Cretaceous and the significance of this biota for understanding the evolution of major invertebrate lineages.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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 - Drawings and processing images
 - Reference nomenclature and cladistic phylogeny

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2023.10.047>.

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AUTHOR CONTRIBUTIONS

D.A. discovered and prepared the material, conceived the project, and prepared the illustrations. D.A., A.N., D.H., and M.S.E. wrote the paper. All authors contributed to the discussions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Studied material		
Holotype, male	Lower Cretaceous amber from Mdeirij- Hammana outcrop, (33°48'13"N, 35°43'45"E, elev. 1,340 m), Caza (= District) Baabda, Central Lebanon ^{10,31–34}	Specimen number 1717A, Azar collection
Paratype, male	Lower Cretaceous amber from Mdeirij- Hammana outcrop, (33°48'13"N, 35°43'45"E, elev. 1,340 m), Caza (= District) Baabda, Central Lebanon ^{10,31–34}	Specimen number 825A, Azar collection
Software and algorithms		
Helicon Focus 8	HeliconSoft	https://www.heliconsoft.com/heliconsoft-products/helicon-focus/
Adobe Photoshop CS6	Adobe	https://www.adobe.com
Adobe Illustrator CS6	Adobe	https://www.adobe.com
PAUP* 4.0a169	PAUP*	https://paup.phylosolutions.com

RESOURCE AVAILABILITY

Lead contact

Further information and requests for methods and materials should be directed to and will be fulfilled by the lead contact, Dany Azar (danyazar@ul.edu.lb)

Materials availability

The studied type material is housed at the Natural History Museum of the Lebanese University, Faculty of Sciences II, Fanar, Lebanon.

Data and code availability

- Request to examine the studied material must be made through the [lead contact](#).
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The type material was collected from the Mdeirij-Hammana outcrop (33°48'13"N, 35°43'45"E, elev. 1,340 m), Caza (= District) Baabda, Central Lebanon. ^{10,35–37} This study is based on taxonomical and morphological investigation on two fossil male culicids. These fossils were compared to all known fossil Cretaceous mosquitoes ^{1,2} and all Recent ones from the different culicid crown-lineages. Then these fossils were integrated in a cladistic phylogeny comprising the closest sister groups of Culicidae and Dixiidae serving as outgroup, in order to better understand their evolutionary history.

METHOD DETAILS

Material preparation

The amber pieces containing the inclusion were manually cut using denticulate shaving blades, and then polished with increasingly finer grade emery papers and diatomite. The specimens were subsequently embedded in Canada balsam between two microscopic coverslips. ³⁸

Microscopic exploration

For examination and photography the following tools were used: a Leitz Laborlux-12 compound microscope, a Nikon SZ10 stereo-microscope, an Olympus CK40 inverted compound microscope, an Olympus IX53 inverted compound microscope, a Leitz wetzlar

compound microscope, Zeiss Stereo Discovery V16 microscope system with incident and transmitted light, Zeiss Axio Imager 2 light microscope with green fluorescence, Zeiss LSM 710 confocal laser scanning microscope (CLSM) using the 488 nm argon laser excitation line, all used devices are equipped with digital cameras.

Drawings and processing images

Line drawings were executed with a camera lucida attached to Leitz Laborlux-12 compound microscope. The figures and illustrations were processed with Helicon Focus 8, Adobe Photoshop CS6, and Adobe Illustrator CS6 softwares.

Reference nomenclature and cladistic phylogeny

Morphological and wing venation nomenclature follows that of Coetzee.³⁹ The morphological character matrix is modified from those presented by Borkent & Grimaldi^{1,40} (see [Tables S1](#) and [S2](#)). The family Dixidae was employed as an outgroup. The analysis was undertaken using the exhaustive search option of PAUP* 4.0a169.

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Supplemental Information

The earliest fossil mosquito

Dany Azar, André Nel, Diying Huang, and Michael S. Engel

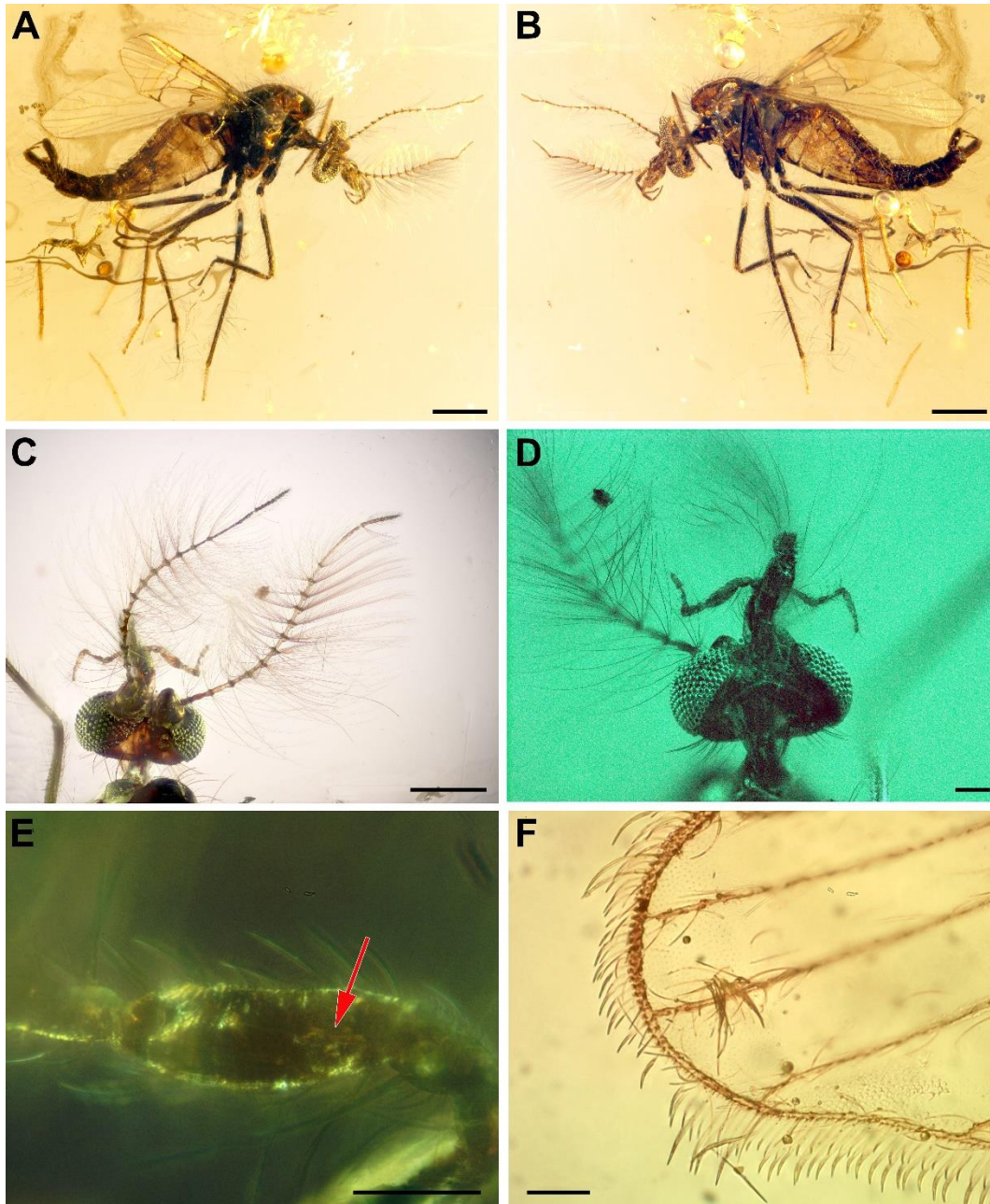


Figure S1. *Libanoculex intermedius* gen. et sp. nov. Related to Figure 1.

(A) and (B) Habitus of paratype, scale bar = 500 microns. (C) Detail of head of holotype, dorsal view, scale bar = 100 microns. (D) Detail of head of holotype, ventral view, observed under laser fluorescent compound microscope, scale bar = 100 microns. (E) Detail of third palpomere with capitate peg on basal fourth of inner surface, scale bar = 50 microns. (F) Detail of wing apex bearing scales, scale bar = 100 microns.

Character	State of character		
1. Posterior margin of wing	with setae (0)	with scales (1)	-
2. R2+3	straight or slightly curved (0)	strongly arched (1, apomorphic)	-
3. R1	extending to near apex of wing (0)	short, ending in C near level of fork of R2 and R3 (1)	-
4. Proboscis	short (0)	moderately long (1); quite elongate (2)	quite elongate (2)
5. Female labrum	relatively short, broad, and dorsal to remaining mouthparts (0)	stylate, partially enclosed by labium (1)	-
6. Lacinia	either reduced or a flattened blade (0)	long, slender, and bearing teeth or fine flattened ridges (1)	-
7. Maxillary palpomere III	short or of moderate length (0)	disproportionately elongate (1).	-
8. Female maxillary palpus	longer than other mouthpart elements (0)	equal in length or shorter than other mouthpart elements (1)	-
9. Clypeus	with setae (0)	without setae (1)	-
10. Maxillary palpus	without scales (0)	with scales (1)	-
11. Female maxillary palpus	pentamerous (0)	tetramerous or with fewer palpomeres, sometimes with vestigial palpomere V (1)	-
12. Legs	without scales (0)	with scales (1)	-
13. R1	without anterior curve (0)	with anterior curve (1)	-
14. Wing veins	without scales (0)	with scales (1)	-
15. Meron	continuously sclerotized with dorsal margin abutting katepimeron (0)	largely membranous with only two small semicircular sclerotized areas along the anterior margin (1).	-

Table S1. List of morphological characters and character-states coded for living and fossil Culicoidea. Related to STAR Methods.

		Character														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Taxon	Dixidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Corethrellidae	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Chaoboridae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	<i>Libanoculex intermedius</i>	1	0	0	1	?	1	0	?	0	0	?	0	0	0	0
	<i>Burmaculex antiquus</i>	1	0	0	1	1	1	1	0	0	0	0	1	1	0	?
	Crown-Culicidae	1	0	0	2	1	1	1	1	1	1	1	1	0	1	0

Table S2. Matrix of morphological character-states coded for extant families of Culicoidea and key fossils. Related to STAR Methods.